Hitachi Chemical

IVD

S TEST Reagent Cartridge Low Density Lipoprotein Cholesterol (LDL)

REF 96313-10 10 S TEST Cartridges
REF 96313-100 10 x 10 S TEST Boxes (100 Tests)

Kit for the determination of Low Density Lipoprotein Cholesterol on HITACHI Clinical Analyzer

2011-11 Rev.0

Intended Use
The S TEST LDL is intended for the quantitative determination of low density lipoprotein cholesterol concentration in serum or heparin plasma using the HITACHI Clinical Analyzer. The S TEST LDL is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

Method
Enzymatic direct method

Test Summary and Explanation
Low Density Lipoprotein (LDL)-Cholesterol is known to be a risk factor for arteriosclerotic disease, especially coronary disease. Determination of total cholesterol has been conventionally and widely used in the diagnosis of hyperlipidemia, which is considered a cause of arteriosclerotic disease. However, it has been reported that ischemic heart disease is more strongly correlated with LDL-Cholesterol than with total cholesterol. [1]

LDL-Cholesterol level is generally calculated from the measurements of total cholesterol, HDL-Cholesterol, and triglycerides using Friedewald's formula, but this calculation method is not sufficient for an accurate determination of LDL-Cholesterol. The reference method for LDL-Cholesterol is the ultracentrifugation method, but the method requires specialized instrumentation and a long measurement time. This makes the reference method difficult to perform in routine laboratory tests, and direct methods are widely used.

Principle of the Test
The presented method using a combination of two surfactants is based on a fact that each lipoprotein reacts with different surfactants depending on their intrinsic physicochemical property. In the first reaction, Surfactant 1 changes the structure of only those lipoproteins other than LDL (i.e., chylomicron [CM], VLDL, and HDL), and the resulting cholesterol is consumed by cholesterol oxidase and cholesterol esterase in a colorless reaction. In the second reaction, the remaining LDL is changed by Surfactant 2, and the resulting cholesterol is consumed in a color reaction.

The first reaction

HDL, VLDL, CM —(Surfactant 1)→ Cholesterol

Cholesterol —(Cholesterol esterase / Cholesterol oxidase)→ H_2O_2

H_2O_2 + 4-Aminoantipyrine —(Peroxidase)→ Colorless

The second reaction

LDL —(Surfactant 2)→ Cholesterol

Cholesterol —(Cholesterol esterase / Cholesterol oxidase)→ H_2O_2

H_2O_2 + 4-Aminoantipyrine + DSBmT —(Peroxidase)→ Purple-red pigment

Reagent Requirements- one cartridge per patient sample

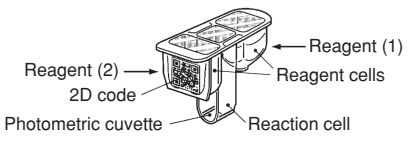
Reagent Composition
The S TEST LDL reagent cartridge has the following composition:

LDL Reagent (1):
- 4-Aminoantipyrine 0.01%
- Cholesterol esterase (Microbial) <2.5 U/mL
- Cholesterol oxidase (Microbial) 1.2 U/mL
- Peroxidase (Horseradish) <1.3 ppg U/mL
- Surfactant 1
- Good's Buffer (pH 6.3)

LDL Reagent (2):
- Surfactant 2
- N,N-bis (4-sulfobutyl)-m-toluidine, disodium salt (DSBmT) 0.04%
- Good's Buffer (pH 6.3)

Preparation and Labeling
LDL reagent is provided in a ready-to-use cartridge. The 2D code label on the front of each cartridge automatically identifies the reagent to the system.

Reagent Cartridge



Precautions
1. This product should be stored to avoid freezing. Frozen reagent should not be used.
2. Reagent exceeding the expiration date should not be used.
3. Avoid direct sunlight during storage and measurement.
4. Avoid all contact with cartridge contents; do not ingest.
5. When handling samples, use disposable gloves to avoid the danger of infection. Do not pipette by mouth.
6. LDL Reagent (1) and (2) contain Proclin, an antiseptic which may be irritating to eyes, skin and mucous membranes.

Disposal Precautions
1. When handling blood and used cartridges, use disposable gloves to avoid the danger of infection.
2. The samples and reagent cartridges should be disposed of as medical waste in accordance with local regulations.

Other Precautions
This product is intended for use on HITACHI Clinical Analyzer. The reagent cartridges should not be used for any other purposes.

Storage and Stability
LDL reagent cartridges are stable until the expiration date shown on the box labels when stored in the refrigerator at 2 – 8 °C.

Specimen Requirements

Patient Preparation
No special patient preparation is required. Collect specimen by standard laboratory technique.

Specimen Collection
1. Use clear, unhemolyzed serum or heparin plasma.
2. Care should be taken to preserve the chemical integrity of the blood specimen from the time it is collected until the time it is assayed (see SPECIMEN HANDLING AND STORAGE).

Specimen Identification
Label each specimen tube with the patient's identification (name and/or number).

Specimen Handling and Storage
Before the measurement, the sample must be brought back to room temperature (15 – 30 °C). If collected serum (plasma) cannot be tested on the same day, store it as follows.

- For testing within 1 week: 2 – 10 °C
- For testing after 1 week or longer: below -20 °C

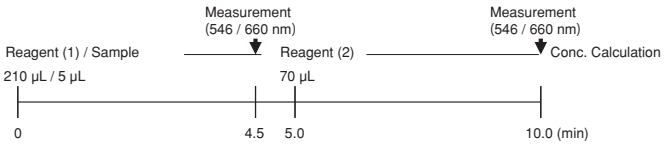
Test Procedure
For complete information on operation, see the User Manual for the HITACHI Clinical Analyzer.

Equipment Required
HITACHI Clinical Analyzer

Reagent Required
S TEST LDL reagent cartridge

Material Required (but not provided)
1. Two levels of controls
2. Sample cups
3. Disposable transfer pipettes
4. Washing water
5. Alkali detergent
6. Waste container

Assay Procedure
Prior to performing each run, check system status to determine the need to replace washing water or empty waste container. See the User Manual for detailed operating instructions.



Presentation of Result
Each Patient Report includes the data and time, sample ID number (as programmed), the test abbreviation, the test results, normal ranges and result flags. For detailed explanations on flags and error messages, refer to the User Manual.

Calibration
Each lot of S TEST LDL cartridges is calibrated by the manufacturer prior to shipment using material traceable to the Beta-Quantification Reference Method (CDC). The 2D code printed on each cartridge provides the analyzer with lot-specific calibration data.

Calculation
LDL-Cholesterol concentration is directly determined by multiplying the change in absorbance of the unknown samples by the calibrator factor on the 2D code. Patient and control results appear on the display.

Quality Control
Users should follow federal, state and local regulatory requirements regarding quality control practices. See instrument manual for procedures on how to run controls. Good laboratory practice includes the use of at least two levels of control material to ensure the test performance. The frequency and limits of QC testing should be determined according to individual laboratory standard QC procedures. Controls should be run at least once every 30 days and:

1. When test results do not match patient symptoms or clinical findings.
2. When using a new lot or shipment of reagents.
3. When laboratory environmental conditions have significantly changed.
4. When training or retraining of personnel occurs.

Reading and Reporting Results

Expected Value

- Reportable range: 8 – 400 mg/dL
- Reference range: 70 – 139 mg/dL [1, 2]
- Optimal: <100 mg/dL, Near optimal: 100 – 129 mg/dL, Borderline high: 130 – 159 mg/dL, High: 160 – 189 mg/dL, Very high: >189 mg/dL [3]
- It is recommended that each laboratory determine the expected values for its particular population.

Interpretation of Results

There may be reactions with non-target substances or interfering reactions. If measured results seem unreliable, repeat the measurement (if necessary after dilution) or try another analytical measurement.

Handling Critical Values

If the result of a sample exceeds the measurement range, dilute the sample with physiological saline solution, and repeat the measurement.

Performance Characteristics

Please note: this assay has not been certified by the Cholesterol Reference Method Laboratory Network (CRMLN), but is traceable to the CRMLN method.

Interference (per CLSI EP7-A2)

The data demonstrated that the LDL test system was not affected by high levels of the following substances at the levels noted:

Hemoglobin: no interference up to 1000 mg/dL
Unconjugated bilirubin: no interference up to 25 mg/dL
Lipemia: no interference up to 614 mg/dL
Ascorbic acid: no interference up to 50 mg/dL
Lack of interference was defined as recoveries between 90% and 110% of the neat value, and assay performance claims were established on the HITACHI Clinical Analyzer by testing two serum pools containing approximately 30 mg/dL and 60 mg/dL LDL-Cholesterol.

Precision (per CLSI EP5-A2)

Four levels of serum samples were assayed 2 times per run, 2 runs per day, for total of 20 days. The precision was found to be:

Level	Mean (mg/dL)	SD (mg/dL)	%CV
1	38	2.0	5.2
2	104	3.6	3.5
3	176	6.0	3.4
4	299	7.9	2.7

n= 80 per level

Precision (POL sites)

Three levels of samples (A, B, and C) were tested by three POL sites, six times a day for five days. The precision estimates are described below.

Site #	Sample	Mean (mg/dL)	Within-run Precision		Total Precision	
			SD (mg/dL)	%CV	SD (mg/dL)	%CV
1	A	41	0.6	1.5	0.7	1.7
2	A	42	0.8	1.9	0.8	1.8
3	A	41	0.8	2.0	0.8	2.0
1	B	109	1.3	1.2	1.4	1.3
2	B	112	1.5	1.4	1.7	1.5
3	B	109	2.3	2.1	2.2	2.0
1	C	183	2.6	1.4	3.1	1.7
2	C	191	2.4	1.2	3.5	1.8
3	C	181	2.6	1.4	3.1	1.7

n = 30 replicates per sample per site

Patient Correlation (POL sites)

A series of 50 or more serum specimens with LDL-Cholesterol values ranging from 25 mg/dL to 283 mg/dL were assayed on the HITACHI Clinical Analyzer at three sites using S TEST LDL (y) and a comparative method as the reference method (x). Linear regression analysis (least squares) yielded the following results.

Site #	n	Range (mg/dL)	Regression Equation	"r"	SE (mg/dL)	CI Slope	CI Intercept
1	52	26 to 274	y = 0.94x +3.8	0.99	8.1	0.90 to 0.99	-1.7 to 9.4
2	50	26 to 283	y = 0.95x +3.6	0.98	9.4	0.89 to 1.01	-3.5 to 10.8
3	51	25 to 276	y = 0.93x +6.4	0.98	8.0	0.89 to 0.98	0.6 to 12.3

CI = 95% confidence interval

Patient Correlation (laboratory site)

A series of 122 serum specimens with LDL-Cholesterol values ranging from 8 mg/dL to 370 mg/dL were assayed on the HITACHI Clinical Analyzer using S TEST LDL (y) and a comparative method as the reference method (x). Linear regression analysis (least squares) yielded the following results.

n	Range (mg/dL)	Regression Equation	"r"	SE (mg/dL)	CI Slope	CI Intercept
122	8 to 370	y = 0.94x + 7.6	0.98	12.5	0.90 to 0.97	3.0 to 12.1

CI = 95% confidence interval

Serum/Plasma Comparison Study

A study was performed to validate the use of heparinized plasma as well as serum for the HITACHI Clinical Analyzer with S TEST LDL. Approximately 40 matched serum/plasma samples that spanned the dynamic range were assayed in singleton and the results were compared using least squares liner regression (plasma = y-axis). The performance characteristics were as follows.

y = 1.01x -4.4
correlation coefficient (r) = 0.99
95% confidence interval of the slope = 0.99 to 1.02
95% confidence interval of the y-intercept -6.4 to -2.3

Detection limit (per CLSI EP17-A)

The detection limit was determined to be 0.8 mg/dL.

Linearity (per CLSI EP6-A)

The assay linearity was determined to be 3 mg/dL to 430 mg/dL.

Reportable Range

8 mg/dL to 400 mg/dL

Routine Maintenance and Troubleshooting

For complete information on operation, see the User Manual for the HITACHI Clinical Analyzer.

Technical Support/ Instrument Service

1. First contact to your local distributor
2. Hitachi Chemical Co., Ltd. (Japan)

Reference

1. Japan Atherosclerosis Society Guideline Investigating Committee for Diagnosis and Treatment of Hyperlipidemia,: Journal of Atherosclerosis and Thrombosis 25, 1-34 (1997)
2. Japanese Journal of Clinical Medicine, 62, Special Issue 18-21 (2004)
3. Third Report of National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Cholesterol in Adults (Adult Treatment Panel III); Executive Summary (2002)
4. CLSI Document EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline
5. CLSI Document EP6-A, Evaluation of Linearity of Quantitative Measurement Procedures, Approved Guideline
6. CLSI Document EP7-A2, Interference Testing in Clinical Chemistry, Approved Guideline
7. CLSI Document EP17-A, Protocols for the Determination of Limits of Detection and Limits of Quantitation, Approved Guideline

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